Evaluation of non-radioactive trivalent DNA probe (LT, ST1a, ST1b) for detecting enterotoxigenic Escherichia coli

PA Chapman, CM Daly

Abstract
Aims: To evaluate a digoxigenin-labelled trivalent DNA probe (LT, ST1a, ST1b) for detecting enterotoxigenic Escherichia coli (ETEC), by comparison with a cell culture assay for detecting LT, individual DNA probes for LT, ST1a and ST1b, and an enzyme immunoassay for detecting ST1.

Methods: A 1268 base pair DNA fragment, containing parts of the genes for E coli heat labile enterotoxin (LT) and heat stable enterotoxins (ST1a and ST1b), was random prime labelled with digoxigenin-dUTP. The labelled DNA was used as a probe in colony hybridisation reactions to examine 180 E coli strains of which 92 had previously been shown by a cell culture assay to produce LT. Six LT negative ST1 positive E coli, 34 Verotoxin producing E coli (VTEC), and 84 organisms from other genera were also examined. All organisms other than VTEC were isolated from travellers returning from abroad with diarrhoea. All E coli strains were tested by cell culture for LT, and were tested by enzyme immunoassay (EIA) for ST1, by the trivalent and individual DNA probes.

Results: All 81 isolates, that on retesting by cell culture were positive for LT, also hybridised with the trivalent and LT probes; 27 of these also enzyme immunoassay (EIA) positive for ST1 of which 24 hybridised with the ST1b probe and three with the ST1a probe. Of 99 isolates, that on retesting by cell culture were negative for LT, all were negative by LT probe and only three were EIA positive for ST1; these three were positive by both trivalent and ST1b probes. Four isolates were positive by the trivalent probe but negative by cell culture and EIA; all four were positive by ST1b probe. Compared with the cell culture assay for LT, the probe had a sensitivity and specificity both of 100%; compared with the EIA for ST1, the probe had a sensitivity of 100% and specificity of 88%.

Conclusions: The trivalent DNA probe is a sensitive, specific, and reliable method for detecting ETEC that should be considered for use by diagnostic microbiology laboratories.

Diarrhoea is the most common health problem in travellers going from developed to less developed countries: about a third develop it during their travels or shortly after returning home. Strains of enterotoxigenic Escherichia coli (ETEC) producing a heat labile enterotoxin (LT) and/or either of two heat stable enterotoxins (ST1a, ST1b) are a major cause of diarrhoea in children and adults in less developed countries. LT positive ETEC are a common cause of diarrhoea in travellers returning to the United Kingdom, both from less developed countries in the tropics, and from the popular Mediterranean holiday destinations of Western Europe.

The aim of this study was to evaluate a recently developed trivalent DNA probe4 for detecting the genes encoding LT, ST1a, and ST1b, by comparison with cell culture assay for LT; a commercial enzyme immunoassay (ST-EIA, Unipath) for detecting ST1, and individual DNA probes for LT, ST1a, and ST1b.

Methods
Test strains included 92 LT positive and 88 LT negative E coli isolated in previous studies4,11 and during subsequent examination of faecal samples for ETEC at Sheffield Public Health Laboratory, and seven strains of E coli known to produce a toxic effect resembling that of LT in one or more of three cell culture assays (Y1, Vero, and CHO). To check the specificity of the probe, six LT negative ST positive E coli (three producing ST1a only and three producing ST1b only) that had been isolated from travellers with diarrhoea returning to Japan and confirmed using DNA probes5 and polymerase chain reaction,8 34 Verotoxin producing E coli (VTEC) of various serogroups isolated in previous studies,9,10 and strains of various other genera isolated from returned travellers with diarrhoea (20 shigellae, 14 salmonellae, 6 vibrios, 4 Plesiomonas shigelloides, 16 Providencia alcalifaciens and 24 Aeromonas hydrophila) were also used. All strains had been stored in nutrient broth with 15% glycerol at −70°C since first isolation. All ETEC strains (except the control strains from Japan) were retested by a Y1 cell assay, a Vero cell assay, and the ST-EIA, at the same time as they were tested using the DNA probes.

For cell culture assays, Y1 cells and Vero cells were grown, maintained, and used to
detect LT, as described before. Known LT positive and LT negative strains were included with each group of tests.

A commercial competitive enzyme immunoassay for ST1 (ST-EIA, Unipath) was performed, as recommended in the supplier's instructions. As an addition to the test procedure, culture supernatant fluids from known ST1 positive and ST negative E. coli strains were included in each batch of tests. 

Further results positive for both trivalent and ST1b probes. Seven strains previously showing a toxic effect on cell culture(s) similar to that of LT, on retesting, produced a toxic effect on Y1 or Vero cells that, unlike LT, was either heat stable, non-neutralisable with anti-cholera toxin, or was progressively cytotoxic; again, none hybridised with the trivalent or LT probes (Table 1). A comparison of the final results obtained with all methods is presented in Table 2. All 81 isolates, that on retesting by cell culture were positive for LT, also hybridised with the trivalent and LT probes; 27 of these 81 were also EIA positive for ST1 of which 24 hybridised with the ST1b probe and three with the ST1a probe. Of 99 isolates, that on retesting by cell culture were negative for LT, all were negative by LT probe and only three were EIA positive for ST1; these three were positive by both trivalent and ST1b probes. Four isolates were positive by the trivalent probe but negative by cell culture and EIA; all four were positive by ST1b probe. The six strains from

### Table 1 Further results on 18 strains of E. coli

<table>
<thead>
<tr>
<th>Y1 cell assay</th>
<th>Vero cell assay</th>
<th>Heat labile</th>
<th>Neutralised by anti-CT</th>
<th>Cytotoxic effect</th>
<th>Trivalent probe</th>
<th>LT Probe</th>
<th>Number of strains</th>
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<tr>
<td><strong>Test strains showing LT-like effect on cell cultures (n = 7)</strong></td>
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<td>Control LT positive strains (n = 2)</td>
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Y1 and Vero cell assays, + = cytopathic effect similar to that of LT; heat labile, + = cytopathic effect destroyed by heating culture supernatant fluids to 65°C; for 30 minutes; neutralised by anti-CT, + = toxic effect neutralised by prior incubation of culture supernatant fluids with anti-cholera toxin, ± = partial neutralisation; cytotoxic effect, + = cytopathic effect progressively cytotoxic over a period of 72 hours.
Japanese travellers all hybridised with the trivalent probe and with the correct monovalent probes. Compared with the cell culture assay for LT the probe had a sensitivity and specificity of 100%; compared with the EIA for ST1 the probe had a sensitivity of 100% and specificity of 88%. None of the VTEC and strains of various other genera hybridised with the trivalent probe.

Discussion

Despite the importance of ETEC as a cause of diarrhoea in returning travellers, few diagnostic microbiology laboratories recognise them. This may in part be due to the difficulties in previously recommended methods, particularly those for detecting ST producers.

Cell culture assays for LT can be simple, economical, and sensitive, and are widely recommended for diagnostic use. Such assays, however, need to be performed in laboratories with specialist cell culture facilities. While the Y1 cell assay is a reliable and sensitive test for LT, other cell culture assays, such as that using CHO cells may be affected by the cytochalasin distending toxin of E coli, and if not investigated further this effect may be confused with that of LT. Previous reports and the present study have highlighted the need for precise confirmation that toxic effects displayed by cell culture are due to LT. LT is antigenic and various immunoassays have been described for its detection; commercial kits for detecting LT have been evaluated, but lack the sensitivity of cell culture. In the present study, 11 isolates previously confirmed as positive for LT by cell culture and a coagglutination assay were found to be negative for LT when restated by cell culture and when examined by the probes. Such discrepancies, due to deletion of fragments of DNA from toxin genes or loss during storage and subculture, of entire plasmids carrying these genes, have been reported by others, such deletions could have occurred in the strains used for this study as they had been stored for varying lengths of time and subcultured several times since first isolation. The EIA used in the present study has been evaluated before, and has given good results, the assay is, however, time consuming to perform and expensive.

The use of a trivalent probe to detect ETEC producing LT, St1a, or ST1b offers a sensitive and reliable means of detecting ETEC. Compared with cell culture the probe had a sensitivity and specificity of 100% for detecting LT; compared with the EIA the sensitivity and specificity for detecting ST1 were 100% and 88% respectively. Although a slightly subjective assessment, LT positive strains gave a strong positive colour reaction using the probe; those producing ST only gave a weaker reaction; Dr Abe and colleagues, who constructed the 1268 base pair sequence, used as a probe in this study, also noted a weak signal in strains producing ST only. However, using the trivalent probe, they correctly identified strains producing ST1a only, which did not react with a biotinylated monovalent ST1a probe used in previous studies. In this study, we found four strains of E coli that were LT negative by cell culture, and which gave a weak reaction with the trivalent probe, characteristic of strains producing ST only; these four strains were negative on repeated testing by EIA for ST1, but did hybridise with the probe for ST1b. Scotland and colleagues found strains that harboured the ST1 gene, but presumably failed to express this, giving negative results for ST in bioassays, and although this could account for our findings, it is possible also that the trivalent probe offers greater sensitivity than the EIA for detecting ST1 positive strains.

Various other organisms have been associated with diarrhoea in travellers returning from abroad, some of which have been reported, usually based on results of cell culture assays, to produce LT-like toxins. No such strains examined in this study hybridised with the trivalent probe.

We conclude that the trivalent DNA probe is a sensitive, specific, and reliable method for detecting ETEC. It should be considered for use by diagnostic microbiology laboratories wishing to examine returning travellers for the presence of these organisms.

We thank Dr Akio Abe of the Kitasato Institute, Tokyo Japan, for supplying the E coli C-600 strain harbouring plasmid pKAD008 and the six strains isolated from Japanese travellers, Unipath Ltd for supplying the ST-EIA kit, and colleagues at Sheffield PHL for their help with this study.

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doi: 10.1136/jcp.46.4.309

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